RESEARCH ARTICLE

Application of selected lactic acid bacteria isolates for bread production without baker's yeast

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Abstract

The isolates of lactic acid bacteria (LAB) from traditional fermented koumiss and homemade dry rye sourdough were examined for their potential to be used in bread production without baker's yeast. Among twenty isolates, three with superior fermentation ability and acidification capability were identified by 16S rRNA gene sequencing. *Leuconostoc mesenteroides* from koumiss, *Levilactobacillus brevis* from 48 h activated rye sourdough, and *Leuconostoc citreum* from rye sourdough extract were singly and mixed (1:1:1) inoculated into liquid sourdoughs and used for bread-making tests. Bread prepared with *Saccharomyces cerevisiae* was used as the control. The substitution of water with whey in LAB fermentation demonstrated lower pH and higher total titratable acidity values in the sourdough. The resulting bread's color parameters, textural properties, and sensory characteristics confirmed the suitability of the selected strains to produce bread without baker's yeast and highlighted the enhancement of new starter varieties.

Keywords Koumiss · Rye sourdough · Isolation · Characterization · *Saccharomyces cerevisiae*

Introduction

Bread is the most common and indispensable food product obtained by baking dough, consisting of at least two components—flour and water. While bread is simple in composition, manufacturers face a growing demand for innovation, driven by consumer preferences for products with enhanced nutritional properties. In particular, there is an increasing trend in the market for food products that exclude ingredients known to cause adverse reactions. The request for yeast-free baked goods is among them, as adverse reactions to products containing baker's yeast (*Saccharomyces cerevisiae*) have been increasing in recent years (De Bellis et al., 2019; Nissen et al., 2020). Some cell wall components of *S. cerevisiae* are recognized as antigens in certain pathologies, such as irritable bowel syndrome, as well as in conditions including ulcerative colitis and Crohn's disease (Rinaldi et al., 2013). Anti-*S. cerevisiae* antibodies (ASCA) are considered a suitable marker for diagnosing Crohn's disease and differentiating it from ulcerative colitis, especially in pediatric populations, since different immune responses to *S. cerevisiae* cell wall phosphopeptidomannans have been observed between them (Brunner et al., 2007; Reese et al., 2006).

Moreover, among patients with hidradenitis suppurativa pathology who adhered to a diet excluding baker's yeast, 70% experienced an improvement in hidradenitis suppurativa symptomatology, with 81% of them following the diet for less than 6 months. Additionally, 87% of patients demonstrated an immediate recurrence of skin lesions less than a week after consuming food containing yeast (Aboud et al., 2020). However, ASCA antibodies have been described in many autoimmune diseases and are highly frequent in patients with type diabetes and COVID-19 (Sakly et al., 2010; Melayah et al., 2022).

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In order to meet the demand for baked goods consumed by individuals with adverse reactions to *S. cerevisiae*, scientific research has been directed toward developing yeast-free baked goods. Several studies have reported selecting LAB strains as alternatives to leavening with baker's yeast (De Bellis et al., 2019; Nissen et al., 2020). Moreover, using LAB in fermentation processes improves the properties of dough and enhances bread's flavor, texture, and nutritional value by increasing the availability of minerals (Teleky et al., 2020). Similarly, LAB causes acidification and lowers the pH, extending bread's microbiological shelf life by controlling and inhibiting spoilage microorganisms (Muhammad et al., 2014). Furthermore, spoilage of bread by mold, which may cause substantial economic losses in the baking industry, is prevented.

Since the metabolic properties of LAB are strain-specific, selecting a proper LAB starter for yeast-free bread is the most crucial step. Fermented traditional foods are rich in LAB sources, and LAB isolates can be considered to assess dough fermenting abilities and bakery applications. Several studies have reported the isolation and characterization of LAB from the fermented dairy beverages koumiss (Rakhmanova et al., 2021; Tang et al., 2020) and rye sourdough (Bartkiene et al., 2019; Digaitiene et al., 2012). However, there is insufficient data surrounding bread-making trials of isolates. The objective of this study was to isolate and select suitable LAB strains (mainly based on their leavening ability) from traditional koumiss made from mare milk and homemade dry rye sourdough. These selected strains were then utilized as starters for the production and characterization of yeast-free doughs and bread.

Materials and methods

Isolation of LAB

Traditionally fermented homemade koumiss and dry rye sourdough were obtained from the local self-employed individual in Bishkek (Kyrgyzstan) and stored at 4 °C until use. Dry rye sourdough was used in activated and nonactivated forms. For activation, 10 g of dry rye sourdough was diluted with 200 mL of deionized sterile water, and 200 g of wheat flour was added and used after 24 and 48 h of cultivation at 30 °C. Additionally, 10 g of dry rye was extracted with 200 mL and used directly without activation. Ten milliliters of each prepared sample and koumiss were separately blended with 90 mL of 0.85% NaCl solution (De Man et al., 1960) and vortexed (Vortex Mixer advanced, Isolab Laborgerate GmbH, Wertheim, Germany). Appropriate decimal dilutions were prepared and plated in triplicate onto media, and counts (CFU/mL) were determined considering the dilution factor used. Lactic acid bacteria were cultured in MRS agar and live, aerobic bacteria were enumerated using Plate Count Agar. Yeasts and molds were selectively isolated and enumerated using Chloramphenicol yeast glucose agar. (Zielińska et al., 2015; Gül et al., 2005).

For the isolation of LAB from four sample preparations, appropriate dilutions were plated on selective MRS agar media. After 48 h of cultivation at 30 °C, colonies of different morphotypes were individually picked and streaked on fresh MRS agar plates by dilution streaking to obtain single settlements. This procedure was repeated to purify the isolates. The pure cultures were stored at −70 °C in MRS broth supplemented with 20% (v/v) glycerol.

Each isolate was first tested for catalase by exposing the cells to a drop of 3% hydrogen peroxide solution. The immediate formation of bubbles indicates the presence of catalase in the cells (Mohd Adnan and Tan, 2007). Gram staining, colony morphology, salt tolerance in MRS broth containing 6.5% NaCl, and pH 9.6 susceptibility tests were performed only for the catalase-negative isolates. A Durham tube was used to evaluate gas production from glucose during fermentation to determine whether the isolate was homo- or heterofermentative.

Selection of LAB

The suitability of selected LAB strains as starters for dough preparation without baker's yeast was checked only with those isolates that produced gas during fermentation. A single colony was transferred into 10 mL of MRS broth to obtain a cell suspension and incubated at 30 °C for 24 h. The cells were harvested by centrifugation at 10,000 rpm for 10 min at 4 °C, washed twice with sterile physiological saline (0.85% NaCl) solution, and resuspended in sterile distilled water, and the cell density was adjusted to ca. 9 log CFU/mL using McFarland Standards and used as inoculum (De Bellis et al., 2019). The dough, with a final cell density of ca. 7 log CFU/g, was prepared according to the procedure described by De Bellis (2019). The wheat flour used had the following proximate composition (%): moisture content—12.69; fat—1.26; saturated fat—0.24; carbohydrates—72.13; sugars—0.99; protein—12.25: salt—0.44 (Berberoğlu Un, Burdur, Turkey). The dough was divided into portions (100 g) and placed in 500 mL graduated cylinders. The fermentation ability (ΔV, mL) in terms of volume increase, as well as pH and total titratable acidity (TTA) after 24 h of incubation at 30 °C and 80% relative humidity, were measured (Li et al., 2022).

16S rRNA sequence analysis

For the initial pool of 18 isolates, only three (K2, 48h3, and E4) exhibited the most promising leavening and acidification capabilities, as identified by 16S rRNA sequencing analysis.

LAB activated in MRS agar medium for 48 h were purified. The EURx GeneMATRIX PCR/DNA Clean-Up Purification Kit (Gdansk, Poland) was used for DNA isolation. A Thermo Scientific NanoDrop 2000 Spectrophotometer (Wilmington, Delaware, USA) was used to control DNA purity. The identification of isolates was analyzed based on 16 s16srDNA gene amplification, using the 27F primer (5′ AGAGTTTGATCMTGGCTCAG 3′) and the 1492R primer (5′ TACGGYTACCTTGTTACGACTT 3′) (Liu et al., 2012). The 35 µL reaction mixture contained 3 µL template DNA, 1× PCR buffer, 1.5 mM MgCl$_2$, 2.0 unit Taq DNA polymerase, 0.2 mM dNTP, and 0.3 µmol of each primer. The PCR amplification procedure was as follows: 5 min at 95 °C, 40 cycles of 45 s at 95 °C, 45 s at 57 °C, 1 min at 72 °C, and then 5 min at 72 °C. The temperature was reduced to 4 °C, and PCR was completed. It was carried out on an automatic thermal cycler (Kyratec, Queensland, Australia). BM Laboratory Systems (Ankara, Turkey) sequenced the purified products. All the isolates' nucleotide sequences of the 16S rRNA gene were analyzed and determined by the BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html) multifunctional sequence alignment editor and were submitted to the National Center for Biotechnology Information (NCBI).

Preparation of liquid sourdoughs and bread making

Bacterial suspensions of three identified strains were prepared as mentioned above and used for bread-making tests. To prepare 50 mL of liquid sourdough, 8.33 g of flour and 29.16 mL of sterile water were singly mixed (1:1:1 ratio) and inoculated with 12.5 mL of bacterial suspension (8 log CFU/mL). Liquid sourdoughs were incubated (Lab Companion SI-300R Shaking Incubator, Massachusetts, USA) at 30 °C for 16 h with constant agitation at 110 rpm.

Bread doughs were prepared in sterile water and sterile whey (pH 4.26 and TTA 0.51 mL 0.1 M NaOH/10 mL) basis with a resulting dough yield (dough weight × 100/flour weight) of 160. To prepare 100 g of dough, 55 g of wheat flour, 1 g of salt (NaCl), 14 mL of sterile water/whey, and 30 mL of liquid sourdough were mixed thoroughly and well kneaded. Doughs with 2% (w/w) baker's yeast and without liquid sourdough were used as controls. Aliquots (100 g) of each dough were placed in rectangular disposable aluminum foil pans and fermented at 25 °C for 6 h (yeast-free) or 3 h (control doughs). Once the dough had risen sufficiently, it was carefully placed into a preheated electric oven that had been set to 190 °C and baked for 20 min. After cooling, three independent breads were taken for subsequent analysis.

pH and TTA

The values of pH and TTA were determined after mixing 10 g of liquid sourdough, dough, or bread with 90 mL of distilled water and homogenizing. Bread samples were read for 10 min before measurements. pH values were measured by direct immersion of the pH electrode (WTW inoLab 9430 IDS Multiparameter Benchtop Meter, Germany) in the samples (AOAC, 2000).

TTA was determined by titration of samples using 0.1 M NaOH in the presence of phenolphthalein as an indicator, and the results were expressed in terms of mL NaOH 0.1 M/10 g sample with Method No 02-31.01 (AACC, 2010).

Characterization of bread

Bread volume was measured according to the AACC 10-05.01 (2010) rapeseed replacement method. After cooling, each bread was placed into a graduated cylinder (V_C). The cylinder was then topped with rapeseed, the bread was removed, and the rapeseed volume was measured (V_R). The bread volume (V_B) was calculated after subtracting V_R from V_C. The same pieces of bread used for measuring volume were weighed on digital scales, W (g). The bread's specific volume (V_S) was calculated according to the following equation.

$$V_s \left(\frac{mL}{g} \right) = \frac{V_B}{W} \tag{1}$$

Bake loss was calculated as the percentage of initial dough weight lost during baking (Ruttarattanamongkol et al., 2011).

$$Bakeloss = \frac{weight_{initial} - weight_{afterbaking}}{weight_{initial}} * 100\% \tag{2}$$

The color was tested using the Chroma Meter (CR-400, Minolta Co., Osaka, Japan) and was reported in the form of a difference ($\Delta E^*_a b$) between the sample and reference (a white ceramic plate, where $L^*_1 = 94.8$, $a^*_1 = 0.4$ and $b^*_1 = 4.16$):

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \tag{3}$$

Texture profile analysis of bread samples was performed using a TA-XTplus Texture Analyzer (Stable Micro Systems, Ltd., Godalming, UK) equipped with a P/5 cylindrical probe (5 mm in diameter) with a test speed of 5 mm/s and 30% specimen deformation. Textural parameters obtained at the end of the measurement were springiness (the rate at which a deformed sample returns to its original size and shape, mm) and surface hardness of the bread (De Bellis et al., 2019).

Sensory evaluation

The external (shape, volume, crust properties, crust color, border properties) and internal properties (taste, texture, flavor, chewing properties, crumb color, pore size) of bread prepared with LAB were evaluated by a panel group composed of ten assessors (IRB # DFE-61286770-302.8.1-692723). External and internal characteristics were evaluated over 30 and 70 points, respectively (Gül et al., 2005).

Statistical analysis

Each method analyzed three replicates of each sample. For the statistical analysis, decimal logarithms of the results were used. One-factor variance analysis was applied to the assessed parameters, and the differences between the groups were evaluated using the Duncan multiple comparison test at a significance level of $\alpha = 0.05$ were performed using the SPSS 16.0 software system.

Results and discussion

Enumeration and isolation of LAB

Traditionally fermented koumiss made from mare milk and homemade dry rye sourdough were used to isolate LAB. As mentioned in the materials and methods section, koumiss was used directly without preparation, and dry rye sourdough was prepared by 3 methods. The viable counts of LAB, total aerobic mesophilic bacteria (TMC), and total yeast and mold (TYMC) are presented in Supplemental Table S1. Microbiological analyses showed the presence of LAB at levels ranging from 5.10 log CFU/mL (nonactivated

extract of rye sourdough) to 9.84 log CFU/mL (48 h activated rye sourdough). The TMC in four samples for isolation ranged from 6.10 to 9.82 log CFU/mL, and TYMC ranged from 2.10 to 6.50 log CFU/mL.

Colony morphology, cell shape, catalase reaction, Gram staining, salt tolerance, pH 9.6 susceptibility, and gas production from glucose were first tested for preliminary separation (Supplemental Table S2). Eighteen gram-positive and catalase-negative microorganisms were presumptively identified as LAB among twenty bacterial isolates obtained. Most isolated strains were coccobacillus-shaped (7 isolates), six were rod-shaped, and the remainder (5 isolates) were cocci. Nine of these isolates produced gas in the carbohydrate fermentation test and were, therefore, deemed to be heterofermentative LAB strains, used for dough fermentation tests.

Selection of LAB

The leavening and acidification capabilities of 9 isolates were evaluated to select LAB strains to be used as starters for dough fermentation (Table 1). Considering the purpose of the study, the most critical parameter considered was the volume increase (ΔV, mL) of dough during fermentation. Doughs inoculated with different isolates showed different leavening capacities during fermentation. Among tested LAB isolates from koumiss, K2 showed remarkable fermenting ability (40.17 mL) and a promising acidifying capacity (pH 4.56) during fermentation. 48h3 isolate from rye sourdough activated for 48 h, and E4 isolate of rye sourdough extract were other strains with a good dough fermentation ability (50.50 and 79.50 mL, respectively), besides giving a good acidity (4.12 and 4.12, respectively) to the doughs. The poor fermenting ability was in the K4 isolate from koumiss.

Table 1 Fermenting ability characteristics of experimental doughs singly inoculated with LAB isolates

LAB isolates	Source	ΔV, mL	pH	TTA, mL NaOH 0.1 M/10 g
K2	Koumiss	40.17 ± 0.26^d	4.56 ± 0.01^c	1.06 ± 0.01^c
K4	Koumiss	30.50 ± 0.45^f	5.10 ± 0.01^a	1.06 ± 0.01^c
K5	Koumiss	35.50 ± 0.45^e	5.06 ± 0.01^b	1.05 ± 0.01^c
48h1	48 h activated rye sourdough	40.50 ± 0.45^d	4.35 ± 0.01^d	0.91 ± 0.01^d
48h3	48 h activated rye sourdough	50.50 ± 0.45^c	4.12 ± 0.01^f	0.82 ± 0.02^e
E2	Non-activated extract of rye sourdough	60.00 ± 0.45^b	4.24 ± 0.02^e	1.11 ± 0.01^b
E3	Non-activated extract of rye sourdough	35.50 ± 0.45^e	4.12 ± 0.01^f	1.55 ± 0.04^a
E4	Non-activated extract of rye sourdough	79.50 ± 0.45^a	4.12 ± 0.01^f	1.10 ± 0.01^b
E5	Non-activated extract of rye sourdough	50.17 ± 0.26^c	4.12 ± 0.01^f	1.11 ± 0.01^b

Data represent means of three independent experiments ± standard deviations

K—isolates from koumiss, 48 h—isolates from 48 h activated rye sourdough, E—isolates fom non-activated extract of rye sourdough

ΔV volume increase, *TTA* total titratable acidity

[a–f]Values in the same column with different letters differ significantly (p < 0.01)

No significant (p > 0.01) differences in ΔV values were found between the doughs inoculated with K5 and E3. TTA of dough inoculated with E3 was the highest. However, the volume increase was relatively low (35.50 mL). Based on these results, K2, 48h3, and E4 isolates were selected and used for further analysis.

Identification of LAB

To confirm the species, 16S rRNA gene sequences of three selected isolates were determined and compared with related bacteria using the BioEdit program at NCBI. According to the obtained results, the K2 isolate was identified as *Leuconostoc mesenteroides* (MT545113.1) with 99.93% similarity, the 48h3 isolate as *Levilactobacillus brevis* (MT611665.1) with a similarity of 99.86% and the third isolate E4 as *Leuconostoc citreum* (MT544904.1), the similarity of which was 100% compared with the 16S rRNA sequences of the type strains. Tegin et al. (2020) identified *Leuconostoc mesenteroides* in koumiss samples collected from three different pastures in Kyrgyzstan. Bartkiene et al. (2019) and Ispirli et al. (2018) isolated *Lactobacillus brevis* and *Leuconostoc citreum* strains from spontaneous rye sourdough manufactured following traditional protocols and rye and rye bran sourdoughs.

Characterization of liquid sourdoughs and bread doughs

The procedure was carried out in two stages of incubation. The first was to prepare a liquid sourdough inoculated with the selected starter (stage I), and the second was aimed at preparing dough for use in bread making (stage II). *Leuconostoc mesenteroides, Levilactobacillus brevis,* and *Leuconostoc citreum* strains were singly (S_{Lm}, S_{Lb}, S_{Lc}) and mixed (S_{mix}) (1:1:1 ratio) as a starter. The results of the characterization of liquid sourdoughs before and after 16 h of incubation are reported in Table 2.

After mixing flour with water and inocula, the pH value of all sourdoughs ranged from 5.67 to 5.86. All strains caused relevant acidification of the sourdoughs by noticeable pH drop and TTA increase after 16 h of fermentation. Consequently, the pH values decreased within the range of 0.96 to 2.11, and the increase in TTA ranged from 1.13 to 5.43 units. After 16 h of fermentation, the lowest pH values (3.63 and 3.62, respectively) were found in sourdoughs inoculated with *Leuconostoc citreum* and mixed starter, with no significant (p > 0.01) differences observed. Similar final pH values have been obtained for sourdoughs inoculated with *Leuconostoc mesenteroides* and *Leuconostoc citreum* (Robert et al., 2006). The highest TTA was observed in S_{Lc} (6.53 mL), followed by S_{mix} (5.40 mL), and the lowest TTA was observed in S_{Lm} (2.10 mL) sourdoughs. De Bellis et al. (2019) reported a pH value of 3.48 and TTA of 4.10 mL after 16 h of incubation at 30 °C for liquid sourdough inoculated with *leuconostoc citreum*.

The microbiological investigation of liquid sourdoughs reported in Table 2 showed initial LAB levels in the range of 6.88 to 7.56 log CFU/mL. After 16 h of fermentation, the final level of LAB levels were abundantly above 7.20 Log CFU/mL for almost all sourdoughs, with the sourdough inoculated with *Leuconostoc citreum displaying* higher cell densities (9.15 log CFU/mL). Corona et al. (2016) reported that LAB behavior remains constant after 16 h of incubation during weekly refreshments in sourdoughs inoculated with *Leuconostoc citreum* (Corona et al., 2016). The TMC

Table 2 Some physicochemical and microbiological characteristics of liquid sourdoughs singly (S_{Lm}, S_{Lb}, S_{Lc}) and mixed (S_{mix}) inoculated with starter strains

Characteristics	Time (h)	S_{Lm}	S_{Lb}	S_{Lc}	S_{mix}
pH	0	5.72 ± 0.01^b	5.86 ± 0.01^a	5.67 ± 0.02^c	5.73 ± 0.01^b
	16	4.76 ± 0.01^a	3.86 ± 0.01^b	3.63 ± 0.02^c	3.62 ± 0.02^c
TTA, mL NaOH 0.1 M/10 g	0	0.97 ± 0.05^c	1.00 ± 0.00^c	1.10 ± 0.09^b	1.40 ± 0.09^a
	16	2.10 ± 0.09^d	3.81 ± 0.01^c	6.53 ± 0.05^a	5.40 ± 0.09^b
LAB, log CFU/mL	0	6.90 ± 0.04^c	7.56 ± 0.06^a	6.88 ± 0.03^c	7.15 ± 0.03^b
	16	7.26 ± 0.22^d	7.59 ± 0.10^c	9.15 ± 0.03^a	8.67 ± 0.05^b
TMC, log CFU/mL	0	8.10 ± 0.15^c	8.36 ± 0.09^a	8.30 ± 0.02^b	8.33 ± 0.06^a
	16	9.98 ± 0.02^c	9.93 ± 0.01^d	10.09 ± 0.01^a	10.03 ± 0.02^b
TYMC, log CFU/mL	0	2.00 ± 0.00^b	2.20 ± 0.15^a	2.10 ± 0.15^{ab}	2.00 ± 0.00^b
	16	2.10 ± 0.15^a	0/ < 1	2.00 ± 0.00^b	0/ < 1

Data represent means of three independent experiments ± standard deviations

TTA total titratable acidity, *LAB* lactic acid bacteria count, *TMC* total mesophilic aerobic bacteria count, *TYMC* total yeast and molds count

[a–d]Values in the same row with different letters differ significantly (p < 0.01)

Lm: Leuconostoc mesenteroides; Lb: Lactobacillus brevis; Lc: Leuconostoc citreum; mix: starter prepared with 1:1:1 ratio of *Leuconostoc mesenteroides: Lactobacillus brevis: Leuconostoc citreum*

of sourdoughs before and after 16 h incubation ranged from 8.10 to 8.36 log CFU/mL and 9.93 to 10.09 log CFU/mL, respectively. Total yeast and mold populations started from an initial level in the 2.00–2.20 log CFU/mL range and remained constant in S_{Lm} and S_{Lc} sourdoughs, while in S_{Lb} and $S_{mix,}$ they were not detected since the acidic environment inhibits spontaneous yeast growth (Martin-Garcia et al., 2023).

Eight different bread doughs, starting with liquid sourdoughs, were prepared on a water (D_{wt-Lm}, D_{wt-Lb}, D_{wt-Lc}, D_{wt-mix}) and whey (D_{cw-Lm}, D_{cw-Lb}, D_{cw-Lc}, D_{cw-mix}) basis (Table 3). Baker's yeast dough (D_{wt-c} and D_{cw-c}), which was made without the addition of sourdough, was considered a control. Final values were obtained for LAB-inoculated doughs after 6 h and for control (baker's yeast alone) after 3 h of fermentation. Both initial and final pH values of doughs made with whey were higher than in dough prepared with water ($p \leq 0.01$), except for dough made with yeast, which had a lower final value. Accordingly, TTA was higher in all dough samples on a whey basis except for D_{wt-Lc}. The pH decreased at the end of fermentation, and the TTA increased in all bread doughs. Notably, the doughs D_{wt-Lc}, D_{cw-Lc}, D_{wt-mix}, and D_{cw-mix} exhibited the highest TTA values, with no significant differences observed between them. As expected, the pH and TTA values of the control dough containing yeast alone varied only slightly on a water basis and whey basis during the fermentation period (pH 5.54–5.37 and 5.04–4.86, TTA 1.00–1.17 and 1.33–1.83, respectively). The observed changes are in accord with the findings of Robert et al. (2006) and De Bellis et al. (2019).

The log CFU/mL of TMC, TYMC, and LAB counts of dough samples before and after fermentation are listed in Table 3. The LAB counts of the doughs after 16 h of fermentation ranged from 6.95 to 8.73 log CFU/mL, with lower values in doughs prepared with whey, even though their initial cell densities were higher. This could be explained by the low pH (or high TTA) tolerance of LAB, which is frequently a growth-limiting factor (Hutkins and Nannen, 1993) since the whey used has a pH of 4.26 and a TTA of 0.51 mL 0.1 M NaOH/10 mL. According to Tsanasidou et al. (2021), whey proteins, such as lactoferrin, immunoglobulins, and lactoperoxidase, with potent antimicrobial activity, may be involved in microbial growth reduction. Moreover, LAB was not detected in bread doughs inoculated with baker's yeast alone. It can be concluded that alcoholic fermentation predominates over lactic acid fermentation in dough prepared with the commonly used concentration of baker's yeast, i.e., 2%, as typically seen in bread production. The total bacterial count was the highest in D_{wt-mix} (10.21 log CFU/mL), followed by D_{wt-Lc} (9.94 log CFU/mL) bread doughs just after mixing, while the highest TMC was in D_{wt-Lc} (8.20 log CFU/mL) at the end of fermentation. Total yeast cell counts were lower than 2.52 log CFU/mL in doughs inoculated with

LAB strains throughout fermentation. Furthermore, yeasts and molds were not detected in bread dough inoculated with *Levilactobacillus brevis*. As expected, the control doughs (D_{wt-c} and D_{cw-c}) showed a very high TYMC.

Bread characteristics

After baking, the bread samples were cooled to room temperature and assessed for quality. The results of the pH, total titratable acidity (mL NaOH 0.1 M/10 g), and the color characteristics of the final bread are reported in Table 4. As expected, the pH values of bread prepared with LAB strains were significantly lower than in the control bread prepared with water B_{wt-c}) ($p \leq 0.05$). The pH values (5.53 for water basis and 5.36 for whey basis) of bread that started with *Leuconostoc mesenteroides* were higher than control bread prepared on a whey basis. A statistically significant difference in pH values between bread made with water and bread made with whey was also observed, with lower values observed in the latter. Differences between them can be attributed to the lower pH of the added whey.

The TTA of B_{wh-mix} (2.57 mL) and B_{wt-mix} (2.27 mL) were significantly higher than the other bread ($p \leq 0.05$), with a ca. 17-fold higher than control B_{wt-c} (0.13 mL) prepared on a water basis. The inoculum *Leuconostoc mesenteroides*-generated bread had lower values of TTA than the pH results. The addition of whey increased the TTA of all breads. Such differences can be attributed to the acidic nature of whey. The results of the pH and TTA values are consistent with microbiological data since the increased acidity of the bread is associated with a decrease in microbial growth.

The three color parameters were determined on three different sides of the bread crust using a colorimeter. As shown in Table 4, the lightness (L*) value of B_{wh-Lm} (81.11) was significantly ($p < 0.05$) higher than that of the other bread and lower than that of the control bread. Similar results were obtained in bread initiated with strains of *Leuconostoc citreum* C2.27 and *Weissella confusa* C5.7 when compared to the control (De Bellis et al., 2019). No significant differences in the green−red component (a*) and blue−yellow component (b*) values were observed among bread B_{wt-mix} and $B_{wh-mix.}$ Color measurements revealed that whey-fortified bread was usually characterized by a brighter, more yellowish crust than water-based bread. According to Tsanasidou (2021), this may be due to vitamin B_2 (riboflavin) in milk, which is 70–80% transferred to whey. All breads exhibited similarly in terms of the ΔE values, except for B_{wh-Lm}, B_{wt-Lb}, and $B_{wh-Lb,}$ which had lower color changes.

Disparities between samples in volume and specific volume were similar for all breads (Table 4). The higher the volume is, the higher the specific volume. The highest volume (154.33 cm^3) and specific volume (2.13 cm^3/g) were in B_{wt-Lc} among LAB-started samples after control ones.

Table 3 Some physicochemical and microbiological characteristics of bread doughs started with the liquid sourdoughs or baker's yeast on water/whey basis

Sample	pH		TTA, mL NaOH 0.1 M/10 g		LAB, log CFU/mL		TMC, log CFU/mL		TYMC, log CFU/mL	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
$D_{wt\text{-}Lm}$	5.65±0.01[a]	4.64±0.02[e]	0.92±0.13[e]	1.50±0.22[c]	7.98±0.02[e]	8.23±0.02[c]	7.80±0.03[g]	7.90±0.05[cd]	2.10±0.16[d]	2.52±0.06[c]
$D_{wh\text{-}Lm}$	5.41±0.01[c]	5.00±0.01[b]	1.17±0.13[cd]	1.50±0.22[c]	8.18±0.03[d]	6.95±0.04[e]	7.01±0.05[h]	7.92±0.06[c]	2.10±0.16[d]	2.10±0.16[e]
$D_{wt\text{-}Lb}$	5.40±0.01[c]	4.57±0.01[f]	1.08±0.13[de]	1.75±0.22[bc]	7.75±0.04[f]	8.08±0.07[d]	7.84±0.06[g]	7.86±0.03[cd]	2.52±0.06[b]	0/<1
$D_{wh\text{-}Lb}$	5.22±0.02[d]	4.72±0.02[d]	1.33±0.13[c]	1.75±0.22[bc]	7.98±0.02[e]	7.00±0.04[e]	8.20±0.16[e]	7.67±0.05[f]	2.00±0.00[d]	2.00±0.00[e]
$D_{wt\text{-}Lc}$	4.97±0.01[f]	4.35±0.01[i]	1.83±0.13[b]	2.17±0.13[a]	8.16±0.01[d]	8.73±0.04[a]	9.94±0.02[b]	8.20±0.16[a]	2.52±0.06[b]	2.10±0.16[e]
$D_{wh\text{-}Lc}$	4.91±0.01[h]	4.52±0.01[g]	1.75±0.22[b]	2.25±0.22[a]	8.77±0.07[b]	8.26±0.22[c]	8.00±0.04[f]	7.80±0.03[de]	2.52±0.06[b]	2.00±0.00[e]
$D_{wt\text{-}mix}$	5.04±0.01[e]	4.33±0.01[j]	1.67±0.13[b]	2.25±0.22[a]	8.95±0.04[a]	8.54±0.02[b]	10.21±0.01[a]	7.92±0.03[c]	2.60±0.00[b]	2.36±0.09[d]
$D_{wh\text{-}mix}$	4.94±0.02[g]	4.44±0.03[h]	2.08±0.13[a]	2.33±0.34[a]	8.49±0.18[c]	8.58±0.02[b]	8.73±0.04[c]	7.90±0.05[cd]	2.30±0.00[c]	2.30±0.00[d]
$D_{wt\text{-}c}$	5.54±0.01[b]	5.37±0.01[a]	1.00±0.22[de]	1.17±0.13[d]	0/<1	0/<1	7.94±0.03[f]	8.10±0.16[b]	7.98±0.02[a]	7.36±0.09[b]
$D_{wh\text{-}c}$	5.04±0.04[e]	4.86±0.01[c]	1.33±0.13[c]	1.83±0.13[b]	0/<1	0/<1	8.37±0.08[d]	7.72±0.05[ef]	8.08±0.20[a]	7.91±0.10[a]

Data represent means of three independent experiments ± standard deviations

TTA total titratable acidity, *LAB* lactic acid bacteria count, *TMC* total mesophilic aerobic bacteria count, *TYMC* total yeast and molds count

[a–j]Values in the same column with different letters differ significantly (p <0.05)

wt: water; wh: whey; *Lm: Leuconostoc mesenteroides*; *Lb: Lactobacillus brevis*; *Lc: Leuconostoc citreum*; mix: dough obtained with 1:1:1 ratio of *Leuconostoc mesenteroides: Lactobacillus brevis: Leuconostoc citreum*; c: control dough

Table 4 Physicochemical, color and textural characteristics of breads

Sample	pH	TTA, mL NaOH 0.1 M/10 g	L*	a*	b*	Delta E	Volume, cm³	Specific volume, cm³/g	Bake loss, %	Surface hardness, g	Springiness, mm
B[wt-Lm]	5.53±0.01[b]	1.07±0.05[h]	65.55±1.17[ef]	2.91±0.58[e]	20.51±1.78[c]	33.66±0.54[ab]	92.33±0.52[c]	1.16±0.01[h]	21.81±1.27[ef]	22.01±0.75[b]	8.93±0.03[b]
B[wh-Lm]	5.36±0.01[c]	1.57±0.05[g]	81.11±1.19[a]	2.96±0.43[e]	15.20±0.97[d]	17.77±1.57[e]	84.33±0.52[i]	1.15±0.01[h]	23.92±0.80[bc]	49.32±0.57[a]	10.65±0.21[a]
B[wt-Lb]	4.88±0.01[e]	1.67±0.05[f]	72.66±2.37[b]	4.70±0.16[cd]	20.70±1.93[c]	27.97±3.02[c]	144.33±0.52[d]	1.93±0.05[d]	24.00±1.10[bc]	14.99±0.32[g]	7.40±1.14[c]
B[wh-Lb]	4.80±0.01[f]	1.97±0.05[d]	78.62±5.02[a]	3.18±1.38[e]	19.82±1.78[c]	22.78±5.01[d]	132.67±1.03[f]	1.86±0.03[e]	25.05±0.70[b]	20.42±0.26[c]	6.68±0.75[cd]
B[wt-Lc]	4.45±0.01[j]	2.07±0.05[c]	67.60±1.83[de]	3.50±0.62[e]	23.10±0.31[b]	33.31±1.58[ab]	154.33±0.52[c]	2.13±0.03[c]	26.44±1.54[a]	13.80±0.25[h]	5.16±2.24[d]
B[wh-Lc]	4.64±0.01[g]	1.97±0.05[d]	72.26±2.23[b]	3.75±0.79[de]	25.27±1.33[a]	31.08±2.51[b]	126.67±1.03[g]	1.69±0.05[g]	21.57±1.92[ef]	16.28±0.73[f]	6.90±1.35[c]
B[wt-mix]	4.51±0.01[h]	2.27±0.05[b]	67.96±1.95[de]	5.54±1.40[c]	25.09±1.35[a]	34.48±1.57[a]	132.33±0.52[f]	1.76±0.01[f]	23.60±0.29[cd]	18.39±0.54[e]	7.06±1.12[c]
B[wh-mix]	4.47±0.01[i]	2.57±0.05[a]	71.89±0.85[bc]	5.25±1.11[bc]	26.28±1.21[a]	32.23±1.49[ab]	136.33±0.52[e]	1.83±0.03[e]	22.65±1.32[de]	19.69±0.75[d]	9.06±2.19[b]
B[wt-c]	5.79±0.01[a]	0.13±0.05[i]	64.49±1.01[f]	8.17±0.55[a]	16.40±0.77[d]	33.62±0.65[ab]	215.67±1.37[b]	2.86±0.03[a]	21.14±0.71[f]	4.52±0.51[j]	6.17±1.01[cd]
B[wh-c]	5.11±0.01[d]	1.77±0.05[e]	69.46±0.90[cd]	6.09±0.22[b]	20.67±0.68[c]	30.80±0.68[b]	225.00±0.89[a]	2.81±0.02[b]	23.08±0.43[cd]	6.18±0.06[i]	7.06±0.07[c]

Data represent means of three independent experiments ± standard deviations

[a–j]Values in the same column with different letters differ significantly (p < 0.05)

wt: water; wh: whey; Lm: *Leuconostoc mesenteroides*; Lb: *Lactobacillus brevis*; Lc: *Leuconostoc citreum*; mix: bread produced with 1:1:1 ratio of *Leuconostoc mesenteroides: Lactobacillus brevis: Leuconostoc citreum*; c: control bread

The lowest physical properties (volume, specific volume) were registered for the bread obtained after fermentation with *Leuconostoc mesenteroides* on water and whey basis with more dense crumbs as shown in Fig. 1. No significant (p ≤ 0.05) differences were found between B[wh-*Lb*] and bread obtained with mixed strains of *Leuconostoc mesenteroides, Levilactobacillus brevis*, and *Leuconostoc citreum* concerning volume and specific volume. In a study by Li et al. (2022), steamed bread prepared from a starter culture of *Saccharomyces cerevisiae* Y5 and *Levilactobacillus brevis* B1 reported a specific volume of 1.70, which closely aligns with our results obtained without the addition of baker's yeast. The authors attributed the increase in specific volume to the gas-holding capacity of the gluten in the dough (Li et al., 2022).

During production, bread loses 10–25% of its original dough weight. Most weight is lost due to moisture loss during bread proofing, baking, and cooling (Kotoki and Deka, 2010). As shown in Table 4, the highest bake loss was in B[wt-*Lc*] (26.44%), followed by B[wh-*Lb*] (24%), and the lowest was in the control bread (21.14%). No significant differences in bread produced with *Leuconostoc mesenteroides* in water and *Leuconostoc citreum* in whey were observed in bake loss. Replacement of water with whey did not affect bake loss in the same way for all breads. Hardness is the force required to compress food between teeth, while springiness is related to bread freshness and elasticity (Olojede et al., 2022). The bread containing the *Leuconostoc mesenteroides* strain prepared in whey required a significantly higher force to break (49.32 N), which is ca. 2 times of bread (22.01 N) in water with the same strain. At the same time, these two samples had a higher springiness (10.65 and 8.93 mm, respectively). The control bread had the lowest hardness (4.52 N), while the bread containing the *Leuconostoc citreum* strain had the lowest springiness (5.16 mm), both of which were prepared with water. The latter had the most insufficient hardness (13.80 N) among bread produced with individual LAB starters. These findings contradict those of Li et al. (2022), who reported lower hardness of steamed bread prepared with starter cultures of *Levilactobacillus brevis* B1 and *Acetobacter tropicalis* in combination with baker's yeast than the control bread prepared with *Saccharomyces cerevisiae* Y5. Water substitution with whey increases the hardness and springiness of all bread. Similar results were reported by Ferreyra et al. (2021), who added 20% whey protein concentrate for the preparation of wheat sourdough bread. Tsanasidou et al. (2021) related this behavior to complexes formed between gluten and lactose contained in whey. A negative relationship between bread hardness and volume was observed, according to other authors' findings (Ferreyra et al., 2021; Zhou et al., 2017).
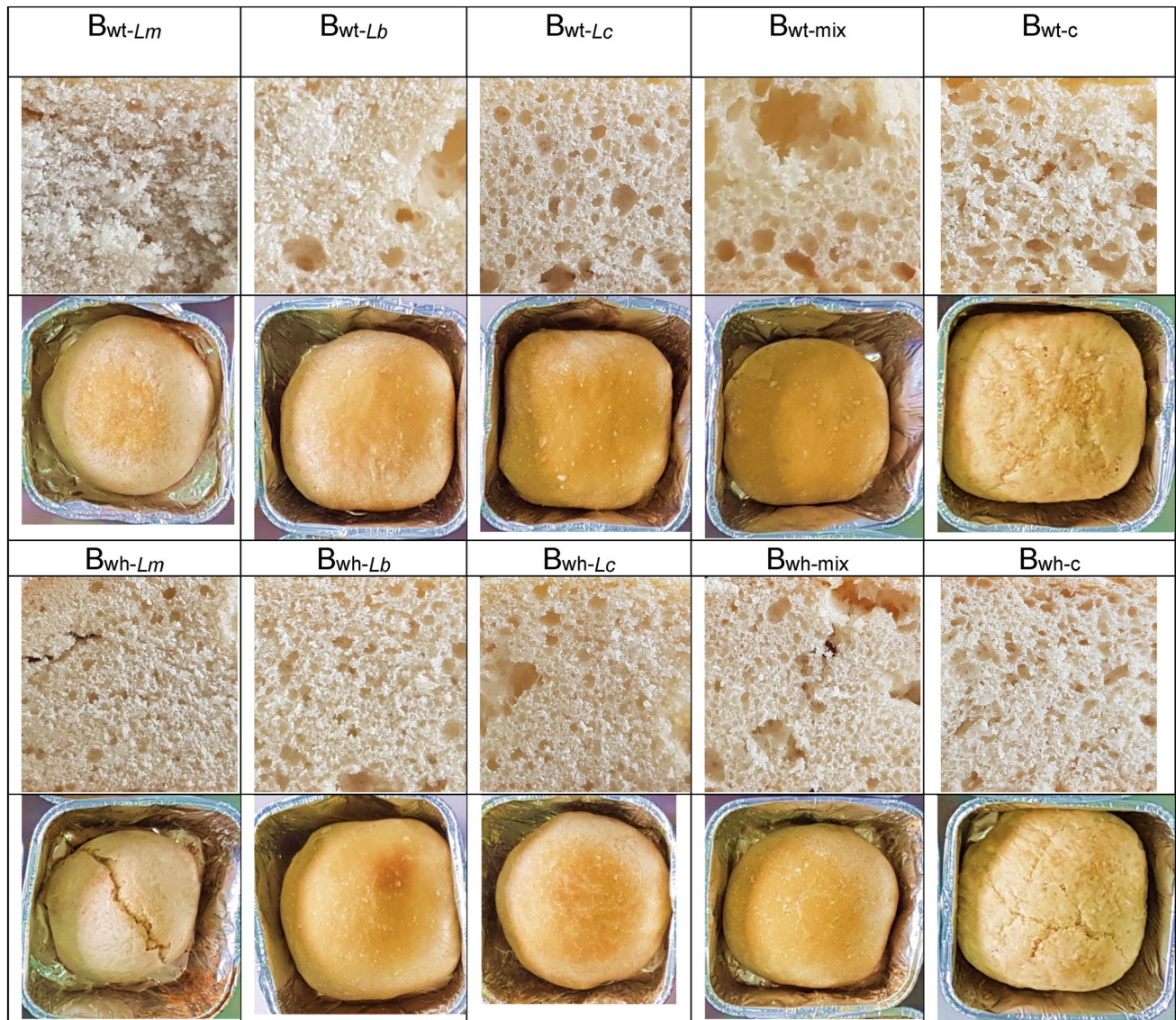
Fig. 1 Images of the cross-sectional and top view of bread (B) made with liquid sourdough inoculated with singly (Lm—*Leuconostoc mesenteroides*; Lb—*Levilactobacillus brevis*; Lc—*Leuconostoc citreum*) or mixed (1:1:1 ratio of *Leuconostoc mesenteroides*, *Levilactobacillus brevis*, and *Leuconostoc citreum*) strains of LAB or with baker's yeast (c) on a water (wt)/whey (wh) basis. Figure 2. Sensory evaluation scores of bread made with liquid sourdough (Lm—*Leuconostoc mesenteroides*; Lb—*Levilactobacillus brevis*; Lc—*Leuconostoc citreum*; mix—1:1:1 ratio of *Leuconostoc mesenteroides*, *Levilactobacillus brevis*, and *Leuconostoc citreum*) or baker's yeast (c) on a water (wt)/whey (wh) basis

Sensory attributes of bread

Individual preference for products is closely linked to the sensory characteristics of food. The results of the sensory evaluation (Fig. 2) showed that the bread (B_{wt-mix}) prepared with a mixed (1:1:1) culture of *Leuconostoc mesenteroides, Levilactobacillus brevis*, and *Leuconostoc citreum* strains in the water had the highest score (28.67) for shape, volume, crust properties, crust color, and crust properties (external properties), followed by bread (B_{wh-Lc}) obtained with *Leuconostoc citreum* (28.20) in whey. B_{wt-Lm}

(23.17) and B_{wt-Lb} (23.17) exhibited the lowest scores for external properties, with no significant differences observed between them. Control bread prepared in whey had the highest score (63.73) in taste, texture, flavor, chewing properties, crumb color, and pore size. The internal property scores of bread prepared with a mixed culture of selected LAB in whey (62.17) and control bread in water (61.83) had no significant differences. The lowest score of internal properties was found in bread obtained after fermentation with *Leuconostoc mesenteroides* in whey (22.40), followed by bread started with the same strain

Fig. 2 Sensory evaluation scores of bread made with liquid sourdough (Lm—*Leuconostoc mesenteroides*; Lb—*Levilactobacillus brevis*; Lc—*Leuconostoc citreum*; mix—1:1:1 ratio of *Leuconostoc mesenteroides*, *Levilactobacillus brevis*, and *Leuconostoc citreum*) or baker's yeast (c) on a water (wt)/whey (wh) basis
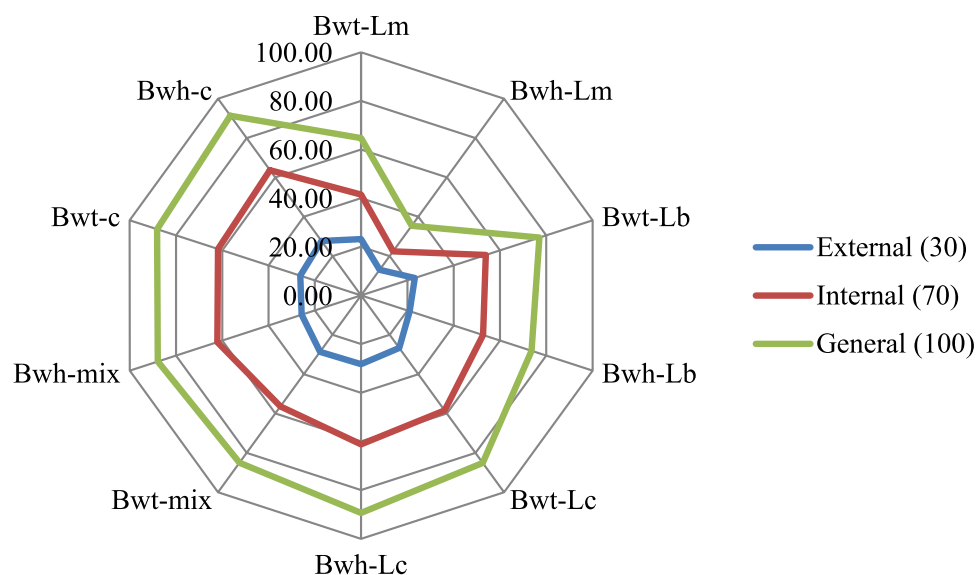
and prepared with water (41.50). The inoculum *Leuconostoc citreum* generated bread exhibited the highest total score (89.37) among bread produced with individual LAB starters, following the control bread prepared with whey. B_{wh-mix} and B_{wt-c} bread had no significant difference in general sensory scores (87.83 and 88.17, respectively). The total value (general properties) for the sensory characteristics of the bread B_{wh-Lm} was the lowest (35.40). Considering that the overall sensory scores ranged from 35.40 to 91.40, the panelists preferred bread made with sourdough containing *Leuconostoc citreum* after control, which may be influenced by habit. Corona et al. (2016) reported that bread contained multiple strain starters of *Leuconosto citreum* PON10079 + *Leuconosto citreum* PON10080 + *Weissella cibaria* PON10030 + *Weissella cibaria* PON10032 compared to single strain starters and mono-species dual strain starters. Gül et al. (2022) reported that inoculum *Lactobacillus brevis* ED25-generated bread was preferred over bread prepared with *Lactobacillus curvatus* N19 and *Weissella cibaria* N9 strains.

According to results of the study, it could be concluded that the selected *Levilactobacillus brevis* and *Leuconostoc citreum* strains allowed bread production with a single-strain starter. At the same time, *Leuconostoc mesenteroides* could be combined with other strains to which symbiotic growth is more inherent for dough fermentation. All strains were suitable for producing bread without baker's yeast with good sensory characteristics and an appearance similar to conventional bread, thus intercepting the current demand from bakers and consumers and reducing the occurrence of possible adverse food reactions. Moreover, using pure starter culture in the bakery production process allows for control and standardization of both the product and the process.

Declarations

References

AACC. Approved Method of the AACC. 11th ed. Method 02-31.01. Titratable Acidity. American Association of Cereal Chemists, St. Paul, MN, USA (2010) https://doi.org/10.1094/AACCIntMethod-02-31.01

AACC. Approved method of the AACC. 11th ed. Method 10-05.01. Guidelines for measurement of volume by rapeseed displacement. American Association of Cereal Chemists, St. Paul, MN, USA (2010) https://doi.org/10.1094/AACCIntMethod-10-05.01

Aboud C, Zamaria N, Cannistrà C. Treatment of hidradenitis suppurativa: Surgery and yeast (*Saccharomyces cerevisiae*)–exclusion diet. The results after 6 years. Surgery. 167: 1012-1015 (2020) https://doi.org/10.1016/j.surg.2019.12.015

AOAC. Official method of analysis of AOAC Intl. 17th ed. Method 943.02. Association of Official Analytical Chemists, Gaithersburg, MD, USA (2000)

Bartkiene E, Lele V, Ruzauskas M, Domig KJ, Starkute V, Zavistanaviciute P, Bartkevics V, Pugajeva I, Klupsaite D, Juodeikiene G, Mickiene R, Rocha JM. Lactic acid bacteria isolation from spontaneous sourdough and their characterization including antimicrobial and antifungal properties evaluation. Microorganisms. 8(1): 64 (2019) https://doi.org/10.3390/microorganisms8010064

Brunner B, Scheurer U, Seibold F. Differences in yeast intolerance between patients with crohn's disease and ulcerative colitis.

Diseases of the Colon and Rectum. 50(1): 83-88 (2007) https://doi.org/10.1007/s10350-006-0749-1

Corona O, Alfonzo A, Ventimiglia G, Nasca A, Francesca N, Martorana A, Moschetti G, Settanni L. Industrial application of selected lactic acid bacteria isolated from local semolinas for typical sourdough bread production. Food Microbiology 59: 43-56 (2016) https://doi.org/10.1016/j.fm.2016.05.006

De Bellis P, Rizzello CG, Sisto A, Valerio F, Lonigro SL, Conte A, Lorusso V, Lavermicocca P. Use of a selected *Leuconostoc citreum* strain as a starter for making a "yeast-free" bread. Foods. 8: 70 (2019) https://doi.org/10.3390/foods8020070

De Man JC, Rogosa D, Sharpe ME. A medium for the cultivation of lactobacilli, Journal of Applied Bacteriology. 23: 130-135 (1960) https://doi.org/10.1111/j.1365-2672.1960.tb00188.x

Digaitiene A, Hansen ÅS, Juodeikiene G, Eidukonyte D, Josephsen J. Lactic acid bacteria isolated from rye sourdoughs produce bacteriocin-like inhibitory substances active against *Bacillus subtilis* and fungi. Journal of Applied Microbiology 112: 732-742 (2012) https://doi.org/10.1111/j.1365-2672.2012.05249.x

Ferreyra LS, Verdini RA, Soazo M, Piccirilli GN. Impact of whey protein addition on wheat bread fermented with a spontaneous sourdough. International Journal of Food Science and Technology. 56: 4738-4745 (2021) https://doi.org/10.1111/ijfs.15052

Gül H, Özçelik S, Sagdiç O, Certel M. Sourdough bread production with lactobacilli and *S. cerevisiae* isolated from sourdoughs. Process Biochemistry 40: 691-697 (2005)

Gül LB, Gül O, Çon AH. Potential of three different lactic acid Bacteria to use as starter culture for production of type II sourdough breadmaking. Journal of Food Measurement and Characterization 16: 3998-4008 (2022) https://doi.org/10.1007/s11694-022-01493-0

Hutkins RW, Nannen NL. pH homeostasis in lactic acid bacteria. Journal of Dairy Science 76: 2354-2365 (1993) https://doi.org/10.3168/jds.s0022-0302(93)77573-6

Ispirli H, Demirbaş F, Yüzer MO, Dertli E. Identification of lactic acid bacteria from spontaneous rye sourdough and determination of their functional characteristics. Food Biotechnology. 32: 222-235 (2018) https://doi.org/10.1080/08905436.2018.1507913

Kotoki D, Deka SC. Baking loss of bread with special emphasis on increasing water holding capacity. Journal of Food Science and Technology 47(1): 128-131 (2010) https://doi.org/10.1007/s13197-010-0008-2

Li H, Hu S, Fu J. Effects of acetic acid bacteria in starter culture on the properties of sourdough and steamed bread. Oil and Gas Science and Technology 5(1): 13-21 (2022) https://doi.org/10.1016/j.gaost.2021.11.003

Liu W, Bao Q, Jirimutu, Qing M, Siriguleng, Chen X, Sun T, Li M, Zhang J, Yu J, Bilige M, Sun T, Zhang H. Isolation and identification of lactic acid bacteria from Tarag in Eastern Inner Mongolia of China by 16S rRNA sequences and DGGE analysis. Microbiology Research 167: 110-115 (2012) https://doi.org/10.1016/j.micres.2011.05.001

Martin-Garcia, A., Riu-Aumatell, M., & Lopez-Tamames, E. Influence of process parameters on sourdough microbiota, physical properties and sensory profile. Food Reviews International. 39: 334-348 (2023) https://doi.org/10.1080/87559129.2021.1906698

Melayah S, Mankaï A, Jemni M, Chaben AB, Ghozzi M, Ben Abdelkrim A, Ach K, Ghariani N, Denguezli M, Benzarti W, Benzarti M, Melayah S, Naija W, Ghedira I. Anti-*Saccharomyces cerevisiae* antibodies in patients with COVID-19. Arab Journal of Gastroenterology. 23: 241-245 (2022) https://doi.org/10.1016/j.ajg.2022.07.001

Mohd Adnan AF, Tan IKP. Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential. Bioresource Technology 98: 1380-1385 (2007) https://doi.org/10.1016/j.biortech.2006.05.034

Muhammad S, Iqra Y, Khan MI, Imran P, Khan MR, Asim S, Khan WA. Lactic acid bacteria in sourdough fermentation; a safe approach for food preservation. Pakistan Journal of Food Sciences. 24: 211-217 (2014)

Nissen L, Rollini M, Picozzi C, Musatti A, Foschino R, Gianotti A. Yeast-free doughs by *Zymomonas mobilis*: evaluation of technological and fermentation performances by using a metabolomic approach. Microorganisms. 8: 792 (2020) https://doi.org/10.3390/microorganisms8060792

Olojede AO, Sanni AI, Banwo K, Michael T. Improvement of texture, nutritional qualities, and consumers' perceptions of sorghum-based sourdough bread made with *Pediococcus pentosaceus* and *Weissella confusa* strains. Fermentation. 8(1): 32 (2022) https://doi.org/10.3390/fermentation8010032

Rakhmanova A, Wang T, Xing G, Ma L, Hong Y, Lu Y, Xin L, Xin W, Zhu Q, Lü X. Isolation and identification of microorganisms in Kazakhstan koumiss and their application in preparing cow-milk koumiss. Journal of Dairy Science. 104: 151-166 (2021) https://doi.org/10.3168/jds.2020-18527

Reese GE, Constantinides VA, Simillis C, Darzi AW, Orchard TR, Fazio VW, Tekkis PP. Diagnostic precision of anti-*Saccharomyces cerevisiae* antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. The American Journal of Gastroenterology. 101: 2410-2422 (2006) https://doi.org/10.1111/j.1572-0241.2006.00840.x

Rinaldi M, Perricone R, Blank M, Perricone C, Shoenfeld Y. Anti-*Saccharomyces cerevisiae* autoantibodies in autoimmune diseases: from bread baking to autoimmunity. Clinical Reviews in Allergy and Immunology. 45: 152-161 (2013) https://doi.org/10.1007/s12016-012-8344-9

Robert H, Gabriel V, Lefebvre D, Rabier P, Vayssier Y, Fontagné-Faucher C. Study of the behavior of *Lactobacillus plantarum* and *Leuconostoc* starters during a complete wheat sourdough breadmaking process. LWT - Food Science and Technology 39: 256-265 (2006) https://doi.org/10.1016/j.lwt.2005.01.013

Ruttarattanamongkol K, Wagner ME, Rizvi SSH. Properties of yeast free bread produced by supercritical fluid extrusion (SCFX) and vacuum baking. Innovative Food Science and Emerging Technologies. 12: 542-550 (2011) https://doi.org/10.1016/j.ifset.2011.07.006

Sakly W, Mankaï A, Sakly N, Thabet Y, Achour A, Ghedira-Besbes L, Jeddi M, Ghedira I. Anti-*Saccharomyces cerevisiae* antibodies are frequent in type 1 diabetes. Endocrine Pathology. 21: 108-114 (2010) https://doi.org/10.1007/s12022-010-9118-7

Tang H, Ma H, Hou Q, Li W, Xu H, Liu W, Sun Z, Haobisi H, Menghe B. Profiling of koumiss microbiota and organic acids and their effects on koumiss taste. BMC Microbiology 20(1): 20-85 (2020) https://doi.org/10.1186/s12866-020-01773-z

Tegin RAA, Gonulalan Z, Deidiev A. Lactic acid bacteria diversity of koumiss samples. Bozok Veterinary Sciences. 1: 1-6 (2020)

Teleky BE, Martău AG, Ranga F, Chețan F, Vodnar DC. Exploitation of lactic acid bacteria and baker's yeast as single or multiple starter cultures of wheat flour dough enriched with soy flour. Biomolecules. 10: 778 (2020) https://doi.org/10.3390/biom10050778

Tsanasidou C, Kosma I, Badeka A, Kontominas M. Quality parameters of wheat bread with the addition of untreated cheese whey. Molecules 26: 7518 (2021) https://doi.org/10.3390/molecules26247518

Zhou J, Liu J, Tang X. Effects of whey and soy protein addition on bread rheological property of wheat flour. Journal of Texture Studies. 49(1): 38–46 (2017) https://doi.org/10.1111/jtxs.12275

Zielińska D, Kamińska A, Kołożyn-Krajewska D. Development of tofu production method with probiotic bacteria addition, Journal

of microbiology, biotechnology and food sciences 4: 485–490 (2015) https://doi.org/10.15414/jmbfs.2015.4.6.485-490.